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INVESTIGATION AND MANAGEMENT OF EBOLA VIRUS INFECTION IN NON-HUMAN PRIMATES

Final Report

by

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January 1981

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-80-G-9472

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A140137	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) INVESTIGATION AND MANAGEMENT OF EBOLA VIRUS INFECTION IN NON-HUMAN PRIMATES		5. TYPE OF REPORT & PERIOD COVERED Final Report 1 Jan 80 - 31 Dec 80 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Arie J. Zuckerman		8. CONTRACT OR GRANT NUMBER(s) DAMD17-80-C-9472
9. PERFORMING ORGANIZATION NAME AND ADDRESS London School of Hygiene and Tropical Medicine Keppel Street (Gower Street) London WC1E7HT		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A.3M161102BS03.00. 051
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, Maryland 21701		12. REPORT DATE January 1981 13. NUMBER OF PAGES 11
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

INVESTIGATION AND MANAGEMENT OF EBOLA VIRUS INFECTION

IN NON-HUMAN PRIMATES. II.

Principal Investigators: DR E T W BOWEN

DR D I H SIMPSON

Aims of the Investigation:

To establish the value of immunotherapy with human convalescent antibody following exposure to Ebola virus infection.

Experience in the use of convalescent Ebola antibody for therapy is very limited and has only been successfully reported on one occasion, and this only in combination with human leucocyte interferon (Emond et al., 1977). The results of previous investigations by us on the use of convalescent plasma as specific treatment in the management of Ebola virus infection in non-human primates have been equivocal and in need of clarification. ~~The following~~ All described subsequently investigations were carried out in an effort to shed some light on some of the mechanisms involved.

Materials and Methods

Animals. Cynomolgus monkeys weighing 4.20 Kg - 5.50 Kg were used.

Immune plasma. Human convalescent plasma from a Zaire patient. Plasma supplied by Dr Karl Johnson, CDC:

	IFA Titre
CDC Est. titre	1024
Porton " "	256
Antwerp " "	256

Challenging virus. The original source of virus was human acute-phase blood, E718 from the Zaire outbreak. This was passaged three times in guinea pigs. The virus inoculum was a suspension of guinea pig liver taken during the late febrile stage of the disease.

Challenge dose. Monkeys were inoculated on day 0 with either a 100 or 1000 guinea pig infectious units intraperitoneally.

Immune plasma treatment schedule

Group A. A total of four monkeys (two infected with 100 GPIU and two with 1000 GPIU) were used. 25 ml of convalescent plasma was administered within 30 minutes of infection.

Group B. A total of four monkeys (two infected with a 100 GPIU and two with a 1000 GPIU) were used. 25 ml of convalescent plasma was administered upon onset of fever.

Group C. Two plasma control monkeys.

Group D. Two virus control monkeys.

Blood samples were obtained daily by femoral venepuncture and estimations made of their virus and passive antibody (IFA) levels. Temperatures were recorded daily.

Results

The results are set out in the accompanying Tables 1, 2 and 3. These show that in the group receiving immune plasma within 30 minutes of infection, viraemia was delayed until day four in three out of the four monkeys infected. The mean survival time in this group was slightly prolonged with one of the monkeys having an inapparent (aborted) infection.

Passive antibody at a level of 1/8 was detected during the first 48 hours. This tended to fall off to undetectable levels from day three onwards.

In the group receiving immune plasma upon onset of fever, the virus was detected in the blood on day two reaching peak titres of 10^6 GPIU/0.2 ml of blood by day four. No passive antibody was detected in the blood of these four monkeys. This was not surprising with the level of viraemia and the time of administration of the plasma and the low level of passive antibody achieved in Group A.

TABLE I. Daily temperature °C

Group	Virus Dose GPIU	Monkey No.	0	1	2	3	4	5	6	7	8	9	10	11	12	Weigh Kg
A 25 ml of plasma infused within 30 mins. Ab. titre = 1/256	1000	1	39*	39	39.1	39.4	40.5	39.9 Rash	Dead	))						5.200
		2	38.3*	38	38.5	39.7	39.4	39.5 Rash	39.5	Dead))						5.200
	100	5	38.5*	38.7	38.3	39.1	40.7	40	39.4 Rash	36.6	Dead					4.250
		6	37.9*	37.8	37.9	38.3	38.3	37.8	37.4	38	37.8		37.9	37.8		4.350
B 25 ml of plasma infused upon onset of fever Ab. titre = 1/256	1000	3	38.9	38.8	39.1	39.1	40.3*	Dead Rash	))							4.550
		4	38.6	38.1	38.7	40.2*	39.2 Rash	39	Dead))							4.750
	100	7	38.7	38.6	38.7	38.6	39.4*	39.0 Rash	Dead	))						4.500
		8	38.3	38.7	38.9	38.8	39.4*	39.7	36.1 Rash	Dead))						4.300
Plasma control	0	9	38.8*	38.9	39.1	38.9	38.8	38.6	38.3	38.9	38.8		38.7	38.9		5.500
	0	10	38.3*	38.7	38.7	38.3	38.2	38.1	37.2	38.3	38.2		38.3	38.3		5.400
Virus control	1000	11	38.2	38.3	38.4	38.4	39.4	38.0 Rash	Dead))							4.350
	100	12	38.6	38.2	38.0	38.7	39.2	37.4 Rash	Dead))							4.700

* Day plasma administered

TABLE 2.

Level of viraemia log 10 (days)

Group	Virus Dose GPIU	Monkey No.	1	2	3	4	5	6	7	8
A 25 ml of plasma infused within 30 mins of infection Ab. titre = 1/256	1000	1	< 0.5	< 0.5	< 0.5	3.0	5.0	4.0+		
		2	< 0.5	< 0.5	2.0	5.0	5.0	5.0	≥ 6.0+	
	100	5	< 0.5	< 0.5	< 0.5	3.0	≥ 6.0	≥ 6.0	≥ 6.0	≥ 6.0+
		6	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
B 25 ml of plasma infused upon onset of fever Ab. titre = 1/256	1000	3	< 0.5	1.0	4.0	≥ 6.0*	≥ 6.0+			
		4	< 0.5	2.0	5.0*	≥ 6.0	≥ 6.0	5.0+		
	100	7	< 0.5	0.5	4.0	≥ 6.0*	5.0	≥ 6.0+		
		8	< 0.5	0.5	3.0	≥ 6.0*	≥ 6.0	≥ 6.0	≥ 6.0+	
Virus control group	1000	11	< 0.5	1.0	2.0	≥ 6.0	5.0	≥ 6.0+		
	100	12	< 0.5	2.0	4.0	≥ 6.0	≥ 6.0	≥ 6.0+		

+ Dead

* Day plasma administered

TABLE 3

Level of passive antibody (days) reciprocal of dilution

Group	Virus Dose GPIU	Monkey No.	0	1	2	3	4	5	6	7	8
A 25 ml of plasma infused within 30 mins. Ab. titre = 1/256	1000	1	<4*	8	4	<4	<4	<4			
		2	<4*	8	8	<4	<4	<4	<4		
	100	5	<4*	<4	8	<4	<4	<4	<4	<4	
		6	<4*	8	8	<4	<4	<4	<4	<4	<4
B 25 ml of plasma infused upon onset of fever Ab. titre = 1/256	1000	3	<4	<4	<4	<4	<4*	<4			
		4	<4	<4	<4	<4*	<4	<4			
	100	7	<4	<4	<4	<4	<4*	<4			
		8	<4	<4	<4	<4	<4*	<4	<4		
Plasma control group		9	<4*	8	8	<4	<4	<4	<4	<4	<4
		10	<4*	<4	<4	<4	<4	<4	<4	<4	<4

* Day plasma administered

In the second experiment we infected a group of monkeys with a 100 GPIU of challenging virus and attempted to maintain a level of passive antibody in the blood by repeated infusion of human convalescent plasma at 48 hr. intervals.

Virus dose - 100 Guinea pig infected units.

Treatment schedule

Group A.	Monkey 1) & " 2)	30 ml of plasma administered intravenously within 30 mins. post infection and every 48 hrs. thereafter.
Group B.	Monkey 3) & " 4)	30 ml of plasma administered intravenously 24 hours post infection and every 48 hrs. thereafter.
Group C.	Monkey 5) & " 6)	30 ml of plasma administered intravenously 48 hours post infection and every 48 hrs. thereafter.
Group D.	Monkey 7) & " 8)	30 ml of plasma administered intravenously 72 hours post infection and every 48 hrs. thereafter.
Group E.	Monkey 9) & " 10)	Plasma control group - schedule as for Group A.
Group F.	Monkey 11) & " 12)	Virus control group.

Blood samples were obtained daily by femoral venepuncture for virus and passive antibody (IFA) levels. Temperatures were recorded daily.

Results

The results of daily temperature and levels of passive antibody are set out in Tables 4 and 5. The viraemia results are not yet completed and will be sent later to complete this report.

In Group A, when plasma was administered within 30 minutes of infection, passive antibody was detected at a level of 1/16 (IFA) on day one in both monkeys. This level dropped to a level of 1/4 - 1/8 on day two. A further administration of plasma on day two and four raised and maintained the detectable level of passive antibody to 1/32. Similar levels of passive antibody were obtained in Groups B and C before onset of fever. Despite the presence of passive antibody

TABLE 4. Daily temperature °C

Group	Virus Dose GPIU	Monkey No.	1.9.80 Days 0	1	2	3	4	5	6	7	8	9	Mean survival time
A 30 ml of plasma infused within 30 mins. of infection and every 48 hrs. thereafter	100	1	38.6*	38.7	38.3*	38.9	40.0*	Rash 38.6	+				6
		2	38.2*	38.6	38.6*	38.8	39.1*	39.6	+				
B 30 ml of plasma infused 24 hrs after infection and every 48 hrs thereafter	100	3	38.2	38.6*	38.2	38.3*	40.0	40.2*	+				6.5
		4	37.9	38.6*	37.8	38.0*	39.8	40.3* Rash	39.1	+			
C 30 ml of plasma infused 48 hrs after infection and every 48 hrs thereafter	100	5	37.8	38.7	39.0*	38.3	40.0*	Rash 38.7	+				7
		6	37.6	38.4	37.8*	37.9	37.2*	39	40.0*	38.4	V. sick +		
D 30 ml of plasma infused 72 hrs after infection and every 48 hrs thereafter	100	7	37.8	38.3	38.3	38.7*	38.8	40.0*	Rash 39.2	+			7
		8	37.4	38.4	38.5	38.5*	39.7	39.7*	39.2	+			
E Plasma controls Schedules as for Group A		9	37.8*	37.4	37.8*	37.7	38.1*	38.0	38.2*				Survived
		10	37.3*	38.1	37.7*	38.0	37.8*	37.8	38.1*				
F Virus Controls	100	11	38.7	38.3	39.2	39.1	39.4	40.0	39.0 Rash	38.7	V. sick +		7.5
		12	38.2	38.2	38.1	38.1	37.6	40.2	39.7	V. sick +			

* = Plasma infused

TABLE 5. Level of passive antibody

Group	Virus Dose GPIU	Monkey No.	Days 0	1	2	3	4	5	6	7	8	9
A 30 ml of plasma infused within 30 mins. of infection and every 48 hrs. thereafter	100	1	<4*	16	8*	32	32*	32				
		2	<4*	16	4*	32	32*	32				
B 30 ml of plasma infused 24 hrs after infection and every 48 hrs thereafter	100	3	<4	<4*	8	32*	32	32*				
		4	<4	<4*	8	32*	32	32*		16		
C 30 ml of plasma infused 48 hrs after infection and every 48 hrs thereafter	100	5	<4	<4	<4*	32	16*	8				
		6	<4	<4	<4*	32	32*	32	*	64		
D 30 ml of plasma infused 72 hrs after infection and every 48 hrs thereafter	100	7	<4	<4	<4	<4*	32	32*		16		
		8	<4	<4	<4	<4*	32	32*		8		
E Plasma controls Schedules as for Group A		9	<4*	8	<4*	32	32*	64	*	64		
		10	<4*	4	<4*	32	32*	64	*	64		
F Virus controls	100	11	-	-	-	-	-	-				
		12	-	-	-	-	-	-				

* = Plasma infused

none of the infected monkeys survived.

These results were obviously disappointing and could suggest that there are differences in the quality of the virus host antibody interaction.

Further studies will now be directed towards combined therapy in the form of serotherapy, Ribavirin and possibly interferon.